Metabolic Engineering of Light and Dark Biochemical Pathways in Wild-Type and Mutant *Synechocystis* PCC 6803 Strains for Maximal, 24-Hour Production of Hydrogen Gas

Roger L. Ely (ely@engr.orst.edu)

Department of Biological & Ecological Engineering, Oregon State University, Corvallis, OR 97331

ABSTRACT

This talk will describe results from an ongoing GTL project in which we are using the cyanobacterial species *Synechocystis* PCC 6803 to address two main factors affecting H_2 production in PCC 6803: NADPH availability and O_2 sensitivity. H_2 production in PCC 6803 requires that the NADP pool be highly reduced, which can be problematic because several metabolic pathways potentially can act to raise or lower NADPH levels. Also, the [NiFe]-hydrogenase (H_2ase) in PCC 6803 is reversibly inactivated at very low O_2 levels due to binding of O_2 at the active site. Largely because of this O_2 sensitivity and the requirement for high NADPH levels, much of the overall H_2 production occurs under anoxic conditions in the dark, supported by breakdown of glycogen or other organic substrates accumulated during photosynthesis. Also, other factors, such as N or S limitation, pH changes, presence of other substances, or deletion of particular respiratory components, can affect light or dark H_2 production. Therefore, we have used H_2 production profiling and metabolic flux analysis to examine light and dark H_2 production under a number of culture conditions with wild-type (WT) PCC 6803 cells and with mutant strains. Also, some of the mutants we have created have shown themselves capable of increased H_2 production. Specific project tasks are as follows:

1. Evaluate the effects of various culture conditions (N, S, or P limitation; light/dark; pH; exogenous organic carbon) on H_2 production profiles;
2. Conduct metabolic flux analyses for enhanced H_2 production profiles using selected culture conditions and inhibitors of specific pathways;
3. Create PCC 6803 mutant strains with modified H_2ases exhibiting increased O_2 tolerance and greater H_2 production;
4. Integrate enhanced H_2ase mutants and culture and metabolic factor studies to maximize 24-hour H_2 production.